

Abcg1 Cas9-CKO Strategy

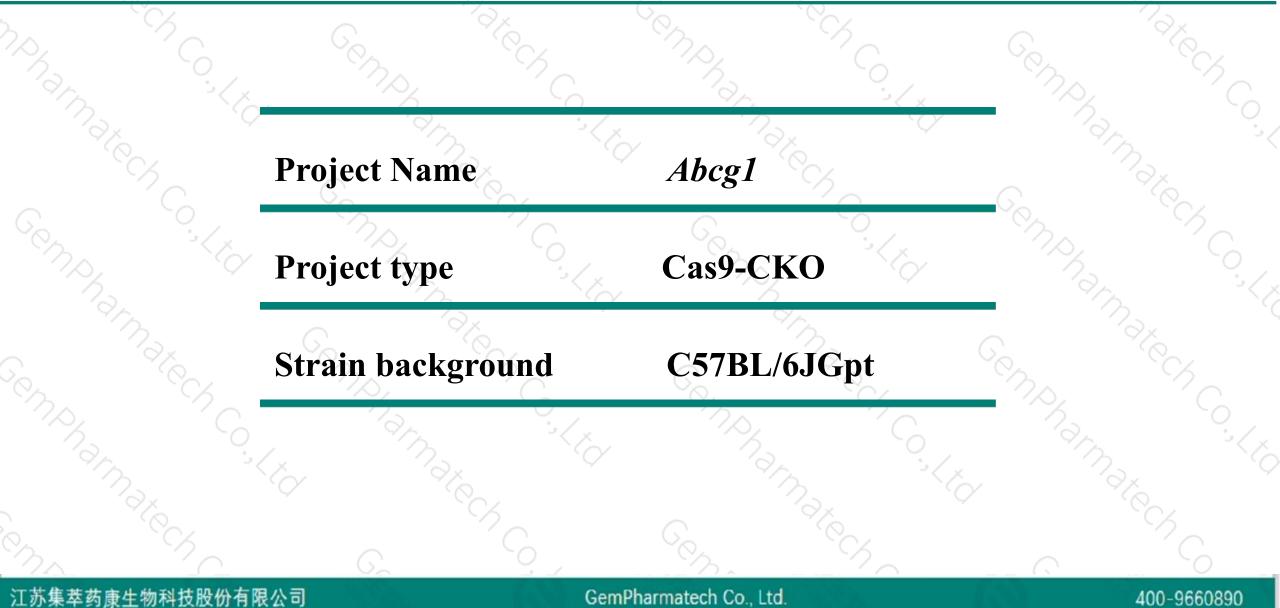
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2019-9-28

Project Overview

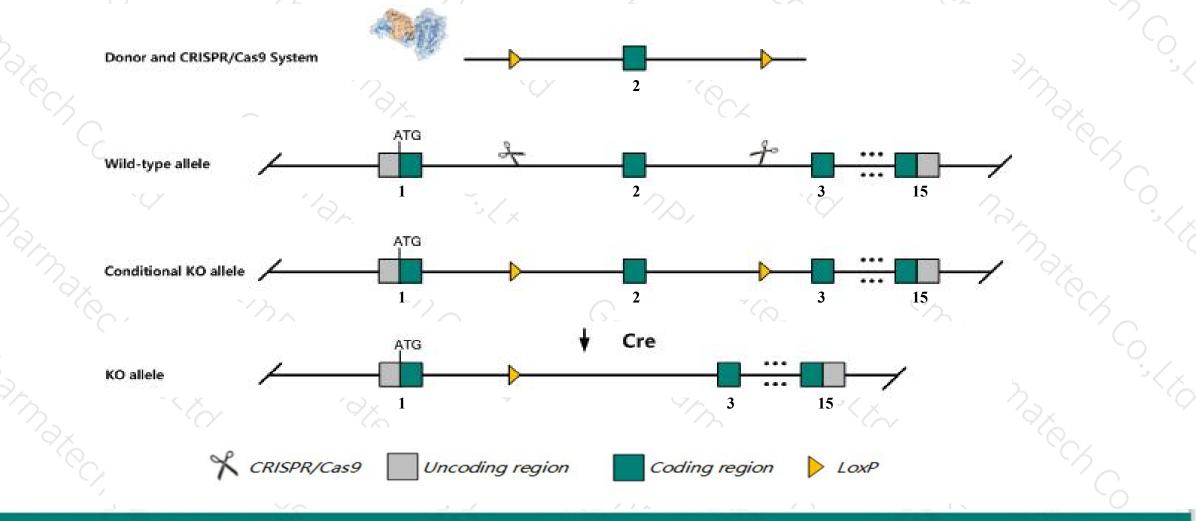




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Abcg1* gene. The schematic diagram is as follows:



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The *Abcg1* gene has 5 transcripts. According to the structure of *Abcg1* gene, exon2 of *Abcg1-201* (ENSMUST00000024829.7) transcript is recommended as the knockout region. The region contains 244bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Abcg1* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit abnormal lipid homeostasis, increased numbers of multiple immune cell types, and abnormal response to a high fat diet.
- The *Abcg1* gene is located on the Chr17. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



Abcg1 ATP binding cassette subfamily G member 1 [Mus musculus (house mouse)]

Gene ID: 11307, updated on 12-Aug-2019

Summary

Official Symbol Abcg1 provided by MGI Official Full Name ATP binding cassette subfamily G member 1 provided by MGI Primary source MGI:MGI:107704 See related Ensembl:ENSMUSG0000024030 Gene type protein coding RefSeg status REVIEWED Organism Mus musculus Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as Abc8: White: AW413978 Summary The protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the White subfamily. The human protein is involved in macrophage cholesterol efflux and may regulate cellular lipid homeostasis in other cell types. [provided by RefSeq, Jul 2008] Expression Broad expression in thymus adult (RPKM 31.5), lung adult (RPKM 23.3) and 16 other tissues See more Orthologs human all

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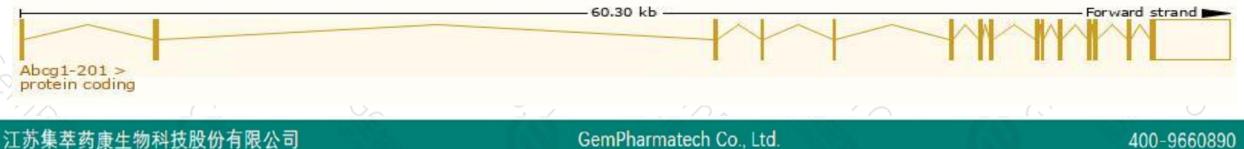
Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

Name 🖕	Transcript ID	bp 👙	Protein 🖕	Biotype 💧	CCDS 🖕	UniProt 💧	Flags	
Abcg1-201	ENSMUST0000024829.7	5832	<u>666aa</u>	Protein coding	<u>CCDS28602</u> &	<u>Q0VDW9</u> & <u>Q64343</u> &	TSL:1 GENCODE basic APPRIS P1	
Abcg1-205	ENSMUST00000236427.1	1372	<u>104aa</u>	Protein coding	-5	<u>A0A494B9K1</u> 료	GENCODE basic	
Abcg1-204	ENSMUST00000236391.1	912	<u>257aa</u>	Protein coding	-	<u>A0A494B9N8</u> 료	CDS 3' incomplete	
Abcg1-203	ENSMUST00000236241.1	644	<u>146aa</u>	Protein coding	-	A0A494BA50	CDS 5' incomplete	
Abcg1-202	ENSMUST00000139013.1	1085	No protein	Retained intron	8	-	TSL:1	

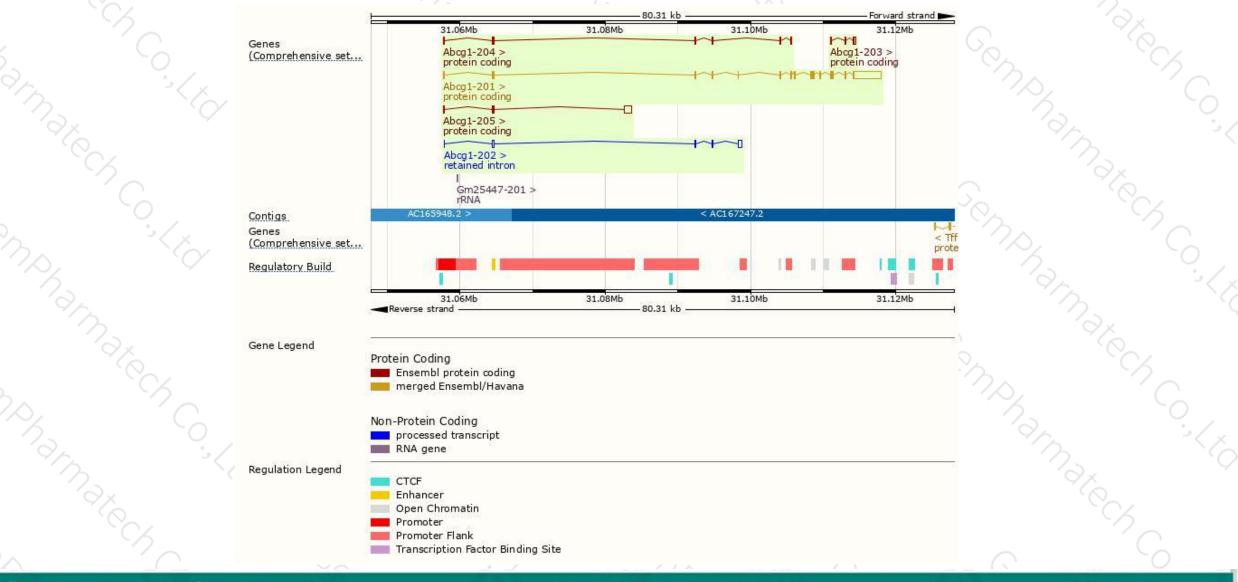
The strategy is based on the design of Abcg1-201 transcript, The transcription is shown below



Genomic location distribution



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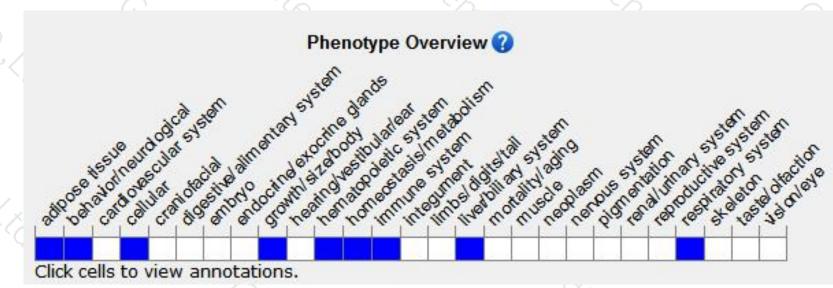
Protein domain



Der C	ENSMUSP00000024 Transmembrane heli Low complexity (Seg) TIGRFAM	Pigment precursor permease/Protein ATP-binding cassette sub-family G	ADD AL
Marmar.	Superfamily SMART	P-loop containing nucleoside triphosphate hydrolase AAA+ ATPase domain	í G
	Pfam. PROSITE profiles	ABC transporter-like ABC-2 type transporter ABC transporter-like	
Senpharp	PROSITE patterns PANTHER	ABC transporter, conserved site PTHR19241:SF211 PTHR19241	6
	Gene3D CDD	3.40.50.300 cd03213	
Sond -	All sequence SNPs/i	Sequence variants (dbSNP and all other sources)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Mpharma	Variant Legend	frameshift variant missense variant splice region variant synonymous variant	Xech of the second seco
SA	Scale bar	0 60 120 180 240 300 360 420 480 540 600 666	6
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Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Mice homozygous for a knock-out allele exhibit abnormal lipid homeostasis, increased numbers of multiple immune cell types, and abnormal response to a high fat diet.



If you have any questions, you are welcome to inquire. Tel: 400-9660890



